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=> d his ful

     FILE 'HCAPLUS' ENTERED AT 14:47:48 ON 16 MAY 2005
                  E FESIK STEPHEN W/AU
L8             226 SEA ABB=ON  ("FESIK S W"/AU OR "FESIK STEPHEN"/AU OR "FESIK
                   STEPHEN W"/AU OR "FESIK STEPHEN WALTER"/AU OR "FESIK STEVE"/AU)
                  E PETROS ANDREW M/AU
L9              49 SEA ABB=ON  ("PETROS ANDREW"/AU OR "PETROS ANDREW M"/AU OR
                   "PETROS ANDREW MARK"/AU)
                  E YOON HO SUP/AU
L10             12 SEA ABB=ON  "YOON HO SUP"/AU
                  E NETTESHEIM DAVID G/AU
L11             45 SEA ABB=ON  ("NETTESHEIM D G"/AU OR "NETTESHEIM DAVID"/AU OR
                   "NETTESHEIM DAVID G"/AU)
L12              1 SEA ABB=ON  L8 AND L9 AND L10 AND L11
L13                ANALYZE L12 1-1 CT :       3 TERMS

     FILE 'REGISTRY' ENTERED AT 14:56:01 ON 16 MAY 2005
                  E BCL-2/CN
                  E BCL-XL/CN

     FILE 'HCAPLUS' ENTERED AT 14:57:30 ON 16 MAY 2005
L14             16 SEA ABB=ON  (?MUTANT?(W)?PROTEIN? AND ?FLEX?(W)?LOOP?)
L15             29 SEA ABB=ON  ?MUTANT?(W)?PROTEIN? AND (BCL-2 OR BCL2 OR BCL-XL
                   OR BCLXL)
L16             44 SEA ABB=ON  L14 OR L15

     FILE 'REGISTRY' ENTERED AT 14:59:28 ON 16 MAY 2005
L17              4 SEA ABB=ON  (GLUTAMIC ACID OR ASPARTIC ACID)/CN

     FILE 'HCAPLUS' ENTERED AT 14:59:52 ON 16 MAY 2005
L18              2 SEA ABB=ON  L16 AND (L17 OR (?GLUTAMIC? OR ?ASPARTIC?)(W)?ACID?
                   ); 2 cits from CA Plus

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO' ENTERED AT
     15:00:49 ON 16 MAY 2005
L19             12 SEA ABB=ON  L18
L20             10 DUP REMOV L19 (2 DUPLICATES REMOVED) 10 cits from other d.b.'s
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=> d que stat l18
L14          16 SEA FILE=HCAPLUS ABB=ON  (?MUTANT?(W)?PROTEIN? AND ?FLEX?(W)?LO
                OP?)
L15          29 SEA FILE=HCAPLUS ABB=ON  ?MUTANT?(W)?PROTEIN? AND (BCL-2 OR
                BCL2 OR BCL-XL OR BCLXL)
L16          44 SEA FILE=HCAPLUS ABB=ON  L14 OR L15
L17           4 SEA FILE=REGISTRY ABB=ON  (GLUTAMIC ACID OR ASPARTIC ACID)/CN
L18           2 SEA FILE=HCAPLUS ABB=ON  L16 AND (L17 OR (?GLUTAMIC? OR
                ?ASPARTIC?)(W)?ACID?)

=> d ibib abs l18 1-2

L18  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         1998:15577  HCAPLUS
DOCUMENT NUMBER:          128:98539
TITLE:                    Recombinant preparation of mutants of cell death
                          regulator protein bcl-2 and their
                          uses
INVENTOR(S):              Korsmeyer, Stanley J.
PATENT ASSIGNEE(S):       Washington University, USA
SOURCE:                   U.S., 85 pp., Cont.-in-part of U.S. Ser. No. 112,208,
                          abandoned.
                          CODEN: USXXAM
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   4
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
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     US 5700638            A     19971223    US 1994-248819          19940525
     US 5691179            A     19971125    US 1993-112208          19930826
     CA 2170143            AA    19950302    CA 1994-2170143         19940824
     CA 2170143            C     20011030
     WO 9505750            A1    19950302    WO 1994-US9701          19940824
         W:  AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI,
             GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN,
             MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US,
             UZ, VN
         RW: KE, MW, SD, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC,
             NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
     AU 9476049            A1    19950321    AU 1994-76049           19940824
     AU 688368             B2    19980312
     EP 722275             A1    19960724    EP 1994-926038          19940824
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
     BR 9407583            A     19970107    BR 1994-7583            19940824
     JP 09502088           T2    19970304    JP 1994-507779          19940824
     NZ 329731             A     20000929    NZ 1994-329731          19940824
     JP 2000336100         A2    20001205    JP 2000-132104          19940824
     US 5856171            A     19990105    US 1994-337646          19941110
     US 5834209            A     19981110    US 1996-661479          19960610
     US 5942490            A     19990824    US 1997-856531          19970514
     US 5955595            A     19990921    US 1997-856034          19970514
     US 6184202            B1    20010206    US 1997-927326          19970911
     US 6500626            B1    20021231    US 1999-379820          19990824
     US 2003096367         A1    20030522    US 2002-277693          20021022
PRIORITY APPLN. INFO.:                       US 1993-112208      B2  19930826
                                             US 1994-248819      A   19940525
                                             JP 1994-507779      A3  19940824
```

NZ 1994-271929	A1 19940824
WO 1994-US9701	W 19940824
US 1994-333565	A3 19941031
US 1994-337646	A3 19941110
US 1997-856034	A3 19970514
US 1999-379820	A1 19990824

AB Disclosed are methods for preparing a mutant of **bcl-2** protein that lacks cell death repressor activity and Bax-binding activity by substitution or deletion in the BH1 or BH2 domain. Also disclosed are methods of identifying candidate **bcl-2**-modulating agents that interfere heterodimerization between **bcl-2** protein and Bax protein. Also disclosed are methods of identifying **bcl-2 mutant proteins**, and of inhibiting the cell death repressor activity of **bcl-2**.

L18 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER:	1996:717465 HCAPLUS
DOCUMENT NUMBER:	126:3743
TITLE:	Proposal for new catalytic roles for two invariant residues in Escherichia coli ribonuclease HI
AUTHOR(S):	Kasiwagi, Tatsuki; Jeanteur, Denis; Haruki, Mitsuru; Katayanagi, Katsuo; Kanaya, Shigenori; Morikawa, Kosuke
CORPORATE SOURCE:	Protein Eng. Res. Inst., Osaka, 565, Japan
SOURCE:	Protein Engineering (1996), 9(10), 857-867 CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER:	Oxford University Press
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Three mutants of E. coli RNase HI, in which an invariant acidic residue, Asp-134, was replaced, were crystallized, and their 3-dimensional structures were determined by x-ray crystallog. Mutant D134A was completely inactive, whereas the other 2 mutants, D134H and D134N, retained 59 and 90% of the activity of the wild-type enzyme, resp. The overall structures of these 3 **mutant proteins** were identical with that of the wild-type enzyme, except for local conformational changes of the **flexible loops.** The RNase H family has a common active site, which is composed of 4 invariant acidic residues (Asp-10, Glu-48, Asp-70, and Asp-134 in E.coli RNase HI), and their relative positions in the mutant, even including the side-chain atoms, are almost the same as those in the wild-type enzyme. The positions of the δ-polar atoms at residue 134 in the wild-type enzyme, as well as in mutants D134H and D134N, coincided well with each other. They were located near the imidazole side-chain of His-124, which is assumed to participate in the catalytic reaction, in addition to the 4 invariant acidic residues. Combined with the pH profiles of the enzymic activities of the 2 other mutants, H124A and H124A/D134N, the crystallog. results allow the authors to propose a new catalytic mechanism of RNase H, which includes roles for Asp-134 and His-124.

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=> d que stat l20
L14          16 SEA FILE=HCAPLUS ABB=ON  (?MUTANT?(W)?PROTEIN? AND ?FLEX?(W)?LO
                OP?)
L15          29 SEA FILE=HCAPLUS ABB=ON  ?MUTANT?(W)?PROTEIN? AND (BCL-2 OR
                BCL2 OR BCL-XL OR BCLXL)
L16          44 SEA FILE=HCAPLUS ABB=ON  L14 OR L15
L17           4 SEA FILE=REGISTRY ABB=ON  (GLUTAMIC ACID OR ASPARTIC ACID)/CN
L18           2 SEA FILE=HCAPLUS ABB=ON  L16 AND (L17 OR (?GLUTAMIC? OR
                ?ASPARTIC?)(W)?ACID?)
L19          12 SEA L18
L20          10 DUP REMOV L19 (2 DUPLICATES REMOVED)

=> d ibib abs l20 1-10

L20  ANSWER 1 OF 10  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
ACCESSION NUMBER:    2004344949  EMBASE
TITLE:               Insertion of foreign T cell epitopes in human tumor
                     necrosis factor α with minimal effect on protein
                     structure and biological activity.
AUTHOR:              Nielsen F.S.; Sauer J.; Backlund J.; Voldborg B.; Gregorius
                     K.; Mouritsen S.; Bratt T.
CORPORATE SOURCE:    F.S. Nielsen, Pharmexa A/S, Kogle Alle 6, DK-2970 Horsholm,
                     Denmark. fn@pharmexa.com
SOURCE:              Journal of Biological Chemistry, (6 Aug 2004) Vol. 279, No.
                     32, pp. 33593-33600.
                     Refs: 30
                     ISSN: 0021-9258  CODEN: JBCHA3
COUNTRY:             United States
DOCUMENT TYPE:       Journal; Article
FILE SEGMENT:        029     Clinical Biochemistry
LANGUAGE:            English
SUMMARY LANGUAGE:    English
ENTRY DATE:          Entered STN: 20040909
                     Last Updated on STN: 20040909
AB   To create a human therapeutic vaccine able to circumvent self-tolerance
     against tumor necrosis factor (TNF) α, foreign T helper epitopes
     were inserted into human TNFα, with minimal effect on the native
     three-dimensional structure. TNFα variants were screened for
     solubility, structural stability, biological activity, and after
     immunization, for eliciting inhibitory antibodies. The longest and most
     flexible loop in TNFα, also designated loop 3, is
     the only region that is not involved in intra- or intermolecular
     interactions and therefore constitute an attractive insertion site.
     However, the extension of the flexible loop by epitope
     insertions destabilized the TNFα molecule. Therefore, two cysteines
     were introduced to form a stabilizing disulfide bond between loops 2 and
     3. In a second design approach, three TNFα monomers were linked by
     two T cell epitopes and expressed as a single chain TNFa trimer. TNFa
     variants that were expressed as soluble proteins also had a conserved
     tertiary structure, as determined by circular dichroism. The biological
     activity of the TNFa variants was of the same magnitude as human
     TNFα in cellular assays. Introduction of three separate
     single-point mutations (D143N, A145R, or Y87S) diminished the cytotoxicity
     of the mutated variants 50-800-fold compared with native TNFα.
     Antisera from mice immunized with the different TNFα variants were
     able to cross-react with native human TNFα and to inhibit TNFα
     signaling via the TNF receptors in vitro, suggesting that the structural
     binding epitopes of native human TNFa and thus the native conformation
```

were conserved in the constructed vaccine candidates.

L20 ANSWER 2 OF 10 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2004030523 EMBASE
TITLE: Mono- and multisite phosphorylation enhances **Bcl2** 's antiapoptotic function and inhibition of cell cycle entry functions.
AUTHOR: Deng X.; Gao F.; Flagg T.; May Jr. W.S.
CORPORATE SOURCE: W.S. May Jr., Univ. of Florida Shands Cancer Ctr., Medical Science Building, 1600 SW Archer Road, Gainesville, FL 32610-0232, United States. smay@ufscc.ufl.edu
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2004) Vol. 101, No. 1, pp. 153-158.
Refs: 27
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040220
Last Updated on STN: 20040220

AB **Bcl2** functions to suppress apoptosis and retard cell cycle entry. Single-site phosphorylation at serine 70 (S70) is required for **Bcl2**'s antiapoptotic function, and multisite phosphorylation at threonine 69 (T69), S70, and S87 has been reported to inactivate **Bcl2.** To address this apparent conflict and identify the regulatory role for **Bcl2** phosphorylation in cell death and cell cycle control, a series of serine/threonine (S/T) → glutamate/alanine (E/A) mutants including T69E/A, S70E/A, S87E/A, T69E/S70A/ S87A (EAA), T69A/S70E/S87A (AEA), T69A/S70A/S87E (AAE), T69E/S70E/S87E (EEE), and T69A/S70A/S87A (AAA) was created to mimic or abrogate, respectively, either single-site or multisite phosphorylation. The survival and cell cycle status of cells expressing the phosphomimetic or nonphosphorylatable **Bcl2** mutants were compared. Surprisingly, all of the E but not the A **Bcl2** mutants potently enhance cell survival after stress and retard G(1)/S cell cycle transition. The EEE **Bcl2** mutant is the most potent, indicating a possible cumulative advantage for multisite phosphorylation of **Bcl2** in survival and retardation of G(1)/S transition functions. Because the E-containing **Bcl2** mutants, but not the A-containing mutants, can more potently block cytochrome c release from mitochondria during apoptotic stress, even at times when steady-state expression levels are similar for all mutants, we conclude that phosphorylation at one or multiple sites within the **flexible loop** domain of **Bcl2** not only stimulates antiapoptotic activity but also can regulate cell cycle entry.

L20 ANSWER 3 OF 10 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
ACCESSION NUMBER: 2003:320847 BIOSIS
DOCUMENT NUMBER: PREV200300320847
TITLE: Cleavage of 14-3-3 protein by caspase-3 facilitates Bad interaction with Bcl-x(L) during apoptosis.
AUTHOR(S): Won, Jungyeon; Kim, Doo Yeon; La, Muhnho; Kim, Doyeun; Meadows, Gary G.; Joe, Cheol O. [Reprint Author]
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute

of Science and Technology, Taejon, 305-701, South Korea
cojoe@mail.kaist.ac.kr
SOURCE: Journal of Biological Chemistry, (May 23 2003) Vol. 278, No. 21, pp. 19347-19351. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Jul 2003
Last Updated on STN: 9 Jul 2003
AB The 14-3-3epsilon protein was identified as one of the caspase-3 substrates by the modified yeast two-hybrid system. The cellular 14-3-3epsilon protein was also cleaved in response to the treatment of apoptosis inducers in cultured mammalian cells. Asp238 of the 14-3-3epsilon protein was determined as the site of cleavage by caspase-3. The affinity of the cleaved 14-3-3 **mutant protein** (D238) to Bad, a death-promoting **Bcl-2** family protein, was lower than that of wild type or the uncleavable mutant 14-3-3epsilon protein (D238A). However, Bad associated with the cellular Bcl-x(L) more effectively in human 293T cells co-expressing Bad with the truncated form of the 14-3-3epsilon protein (D238) than in control cells co-expressing Bad with wild type or the uncleavable mutant 14-3-3epsilon protein (D238A). The present study suggests that the cleavage of 14-3-3 protein during apoptosis promotes cell death by releasing the associated Bad from the 14-3-3 protein and facilitates Bad translocation to the mitochondria and its interaction with Bcl-x(L).

L20 ANSWER 4 OF 10 EMBASE
on STN
ACCESSION NUMBER: 2003487736 EMBASE
TITLE: Enhancement of Bik Antitumor Effect by Bik Mutants.
AUTHOR: Li Y.M.; Wen Y.; Zhou B.P.; Kuo H.-P.; Ding Q.; Hung M.-C.
CORPORATE SOURCE: M.-C. Hung, Dept. of Molec. and Cell. Oncology, Unit 79, Univ. TX M. D. Anderson Cancer Ctr., 1515 Holcombe Boulevard, Houston, TX 77030, United States.
mhung@mail.mdanderson.org
SOURCE: Cancer Research, (15 Nov 2003) Vol. 63, No. 22, pp. 7630-7633.
Refs: 13
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040105
Last Updated on STN: 20040105
AB Bik was initially identified as a BH3-domain-only protein that interacts with E1B 19K. Although systemically administered wild-type Bik significantly inhibited tumor growth and metastasis in an orthotopic nude mouse model, the proapoptotic potency of Bik can be modulated by posttranslational phosphorylation. Here, we found that Bik mutants, in which threonine 33 and/or serine 35 were changed to **aspartic acid** to mimic the phosphorylation at these two residues, enhanced their binding affinity with the antiapoptotic proteins Bcl-X(L) and **Bcl-2** and were more potent than wild-type Bik in inducing apoptosis and inhibiting cell proliferation in various human

cancer cells. Bik mutants also suppressed tumorigenicity and tumor-taking rate in a mouse ex vivo model. Moreover, Bik mutant-liposome complexes inhibited tumor growth and prolonged life span more effectively than the wild-type Bik-liposome complex in an in vivo orthotopic animal model. Thus, our results demonstrate that Bik mutant genes, more potent than wild-type Bik, induce cell death and suggest that their inhibition on the growth of various cancers should be explored further.

L20 ANSWER 5 OF 10 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2002-490141 [52] WPIDS
DOC. NO. CPI: C2002-139202
TITLE: New mutant **Bcl-2** proteins derived from a wild type human **Bcl-2** protein, useful in biological assays to identify substances that block the ability of **Bcl-2** to inhibit programmed cell death or apoptosis.
DERWENT CLASS: B04 D16
INVENTOR(S): FESIK, S W; NETTESHEIM, D G; PETROS, A M; YOON, H
PATENT ASSIGNEE(S): (ABBO) ABBOTT LAB
COUNTRY COUNT: 23
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002040530	A2	20020523	(200252)*	EN	36
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR					
W: CA JP MX					
EP 1337555	A2	20030827	(200357)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR					
JP 2004526673	W	20040902	(200457)		59
MX 2003004436	A1	20040501	(200482)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002040530	A2	WO 2001-US45693	20011115
EP 1337555	A2	EP 2001-987213	20011115
		WO 2001-US45693	20011115
JP 2004526673	W	WO 2001-US45693	20011115
		JP 2002-543538	20011115
MX 2003004436	A1	WO 2001-US45693	20011115
		MX 2003-4436	20030520

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 1337555	A2	Based on	WO 2002040530
JP 2004526673	W	Based on	WO 2002040530
MX 2003004436	A1	Based on	WO 2002040530

PRIORITY APPLN. INFO: US 2000-716395 20001120
AN 2002-490141 [52] WPIDS
AB WO 200240530 A UPAB: 20020815
NOVELTY - A **mutant protein**, which is derived from a wild type human **Bcl-2** protein, is new. A sequence of amino acid residues comprising a **flexible loop** from the wild type **Bcl-2** protein is replaced with a

replacement amino acid sequence comprising at least two acidic amino acids. The mutant **Bcl-2** protein comprises a 166 residue amino acid sequence, given in the specification.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an assay for identifying substances that bind to the **Bcl-2** protein, comprising:

(a) providing a candidate substance to be tested;

(b) providing a labeled peptide that is capable of binding tightly to the **mutant protein**;

(c) forming a complex of the labeled peptide with the **mutant protein**;

(d) forming a reaction mixture by contacting the candidate substance with the labeled peptide/**mutant protein** complex;

(e) incubating the reaction mixture to allow the candidate substance to react and displace the labeled peptide; and

(f) determining the amount of labeled peptide that has been displaced from binding to the **mutant protein.**

USE - The protein is useful in biological assays to identify substances that block the ability of **Bcl-2** to inhibit programmed cell death or apoptosis.

Dwg.0/3

L20 ANSWER 6 OF 10 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002413225 EMBASE

TITLE: The proapoptotic BH3-only protein BAD transduces cell death signals independently of its interaction with **Bcl-2.**

AUTHOR: Adachi M.; Imai K.

CORPORATE SOURCE: M. Adachi, The First Dept. of Internal Medicine, Graduate School of Medicine, Sapporo Medical University, Sapporo, Japan. adachi@sapmed.ac.jp

SOURCE: Cell Death and Differentiation, (1 Nov 2002) Vol. 9, No. 11, pp. 1240-1247.
Refs: 33
ISSN: 1350-9047 CODEN: CDDIEK

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 20021202
Last Updated on STN: 20021202

AB The BH3-only protein BAD binds to **Bcl-2** family proteins through its BH3 domain. Recent studies suggest that BAD binds to both **Bcl-2** and Bcl-X(L), however mediates its pro-apoptotic functions through inhibition of BCl-X(L), but not **Bcl-2.** In this paper we addressed this issue using a BAD mutant within the BH3 domain, by substitution of Asp 119 with Gly (BAD(D119G)), which selectively abrogates an ability to interact with **Bcl-2.** Confocal microscopy revealed that mutation of BAD at D119 does not affect BAD targeting to the mitochondrial membrane in serum-starved COS-7 cells. However, co-precipitation assays indicated that, whereas wild-type BAD (BADwt) directly interacts with **Bcl-2** and Bcl-X(L), BAD(D119G) interacts only with Bcl-X(L). Nevertheless both BADwt and BAD(D119G) could introduce apoptosis and diminish the anti-apoptotic effect of **Bcl-2** and Bcl-X(L) in a similar manner in a co-transfection assay. These data thus suggest that Asp119 is a crucial site within the BH3 domain of BAD for

interaction of BAD with **Bcl-2**, but is dispensable for the interaction of BAD with Bcl-X(L), for its targeting to mitochondria, and most importantly, for its pro-apoptotic functions. Thus, we confirm that neutralization of **Bcl-2** function is marginal for BAD-mediated apoptosis.

L20 ANSWER 7 OF 10 EMBASE on STN

ACCESSION NUMBER: 2000022905 EMBASE
TITLE: Effects on general acid catalysis from mutations of the invariant tryptophan and arginine residues in the protein tyrosine phosphatase from Yersinia.
AUTHOR: Hoff R.H.; Hengge A.C.; Wu L.; Keng Y.-F.; Zhang Z.-Y.
CORPORATE SOURCE: A.C. Hengge, Dept. of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300, United States
SOURCE: Biochemistry, (11 Jan 2000) Vol. 39, No. 1, pp. 46-54. Refs: 31
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20000120
Last Updated on STN: 20000120

AB General acid catalysis in protein tyrosine phosphatases (PTPases) is accomplished by a conserved Asp residue, which is brought into position for catalysis by movement of a **flexible loop** that occurs upon binding of substrate. With the PTPase from Yersinia, we have examined the effect on general acid catalysis caused by mutations to two conserved residues that are integral to this conformation change. Residue Trp354 is at a hinge of the loop, and Arg409 forms hydrogen bonding and ionic interactions with the phosphoryl group of substrates. Trp354 was mutated to Phe and to Ala, and residue Arg409 was mutated to Lys and to Ala. The four mutant enzymes were studied using steady state kinetics and heavy-atom isotope effects with the substrate p-nitrophenyl phosphate. The data indicate that mutation of the hinge residue Trp354 to Ala completely disables general acid catalysis. In the Phe mutant, general acid catalysis is partially effective, but the proton is only partially transferred in the transition state, in contrast to the native enzyme where proton transfer to the leaving group is virtually complete. Mutation of Arg409 to Lys has a minimal effect on the K(m), while this parameter is increased 30-fold in the Ala mutant. The k(cat) values for R409K and for R409A are about 4 orders of magnitude lower than that for the native enzyme. General acid catalysis is rendered inoperative by the Lys mutation, but partial proton transfer during catalysis still occurs in the Ala mutant. Structural explanations for the differential effects of these mutations on movement of the **flexible loop** that enables general acid catalysis are presented.

L20 ANSWER 8 OF 10 EMBASE on STN

ACCESSION NUMBER: 1999264548 EMBASE
TITLE: A structural snapshot of an intermediate on the streptavidin-biotin dissociation pathway.
AUTHOR: Freitag S.; Chu V.; Penzotti J.E.; Klumb L.A.; To R.; Hyre D.; Le Trong I.; Lybrand T.P.; Stenkamp R.E.; Stayton P.S.
CORPORATE SOURCE: T.P. Lybrand, Department of Bioengineering, Box 351750, University of Washington, Seattle, WA 98195, United States.

lybrand@proteus.bioeng.washington.edu
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (20 Jul 1999) Vol. 96, No. 15, pp. 8384-8389.
Refs: 38
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19990812
Last Updated on STN: 19990812

AB It is currently unclear whether small molecules dissociate from a protein binding site along a defined pathway or through a collection of dissociation pathways. We report herein a joint crystallographic, computational, and biophysical study that suggests the Asp-128 → Ala (D128A) streptavidin mutant closely mimics an intermediate on a well-defined dissociation pathway. Asp-128 is hydrogen bonded to a ureido nitrogen of biotin and also networks with the important aromatic binding contacts Trp-92 and Trp-108. The Asn-23 hydrogen bond to the ureido oxygen of biotin is lengthened to 3.8 Å in the D128A structure, and a water molecule has moved into the pocket to replace the missing carboxylate interaction. These alterations are accompanied by the coupled movement of biotin, the flexible binding loop containing Ser-45, and the loop containing the Ser-27 hydrogen bonding contact. This structure closely parallels a key intermediate observed in a potential of mean force-simulated dissociation pathway of native streptavidin, where the Asn-23 hydrogen bond breaks first, accompanied by the replacement of the Asp-128 hydrogen bond by an entering water molecule. Furthermore, both biotin and the **flexible loop** move in a concerted conformational change that closely approximates the D128A structural changes. The activation and thermodynamic parameters for the D128A mutant were measured and are consistent with an intermediate that has traversed the early portion of the dissociation reaction coordinate through endothermic bond breaking and concomitant gain in configurational entropy. These composite results suggest that the D128A mutant provides a structural 'snapshot' of an early intermediate on a relatively well-defined dissociation pathway for biotin.

L20 ANSWER 9 OF 10 EMBASE
on STN
ACCESSION NUMBER: 96282527 EMBASE
DOCUMENT NUMBER: 1996282527
TITLE: Bax can antagonize Bcl-X(L) during etoposide and cisplatin-induced cell death independently of its heterodimerization with Bcl-X(L).
AUTHOR: Simonian P.L.; Grillot D.A.M.; Merino R.; Nunez G.
CORPORATE SOURCE: Dept. of Pathology, Michigan University Medical School,Ann Arbor, MI 48109, United States
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 37, pp. 22764-22772.
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index

LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 961007
Last Updated on STN: 961007

AB Bax, a member of the **Bcl-2** family of proteins, has been shown to promote apoptosis while other members of the family, including Bcl-X(L) and **Bcl-2**, inhibit cell death induced by a variety of stimuli. The mechanism by which Bax promotes cell death is poorly understood. In the present report, we assessed the ability of Bax to antagonize the death repressor activity of Bcl-X(L) during chemotherapy-induced apoptosis in the lymphoid cell line, FL5.12. Expression of wild-type Bax countered the repressor activity of Bcl- X(L) against cell death mediated by VP-16 and cisplatin. We performed site-directed mutagenesis of the BH1, BH2, and BH3 homology regions in Bax to determine the ability of wild-type and mutant Bax to heterodimerize with Bcl- X(L) and to antagonize the protective effect of Bcl-X(L) against chemotherapy-induced apoptosis. Bax proteins expressing alanine substitutions of the highly conserved amino acids glycine 108 in BH1, tryptophan 151 and 158 in BH2, and glycine 67 and **aspartic acid** 68 in BH3 retained their ability to promote chemotherapy-induced cell death that was inhibited by Bcl-X(L) and to form heterodimers with Bcl-X(L). Bax proteins containing deletions of the most highly conserved amino acids in BH1 (Δ102-112) and BH2 (Δ151-159) maintained the ability of Bax to antagonize the death repressor activity of Bcl-X(L) and to associate with Bcl-X(L). However, Bax with BH3 deleted did not form heterodimers with Bcl-X(L), but retained its ability to counter the death repressor activity of Bcl-X(L). These results demonstrate that the conserved BH3, but not BH1 or BH2, homology region of Bax is necessary for its interaction with Bel-X(L) in mammalian cells. Further more, our results indicate that Bax does not require BH1, BH2, BH3, or heterodimerization with Bcl-X(L) to counter the death repressor activity of Bcl-X(L). Therefore, Bax can antagonize Bcl-X(L) during VP-16 and, in a lesser degree, during cisplatin-induced cell death independent of its heterodimerization with Bcl- X(L).

L20 ANSWER 10 OF 10 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 97084791 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8931125
TITLE: Proposal for new catalytic roles for two invariant residues in Escherichia coli ribonuclease HI.
AUTHOR: Kashiwagi T; Jeanteur D; Haruki M; Katayanagi K; Kanaya S; Morikawa K
CORPORATE SOURCE: Protein Engineering Research Institute, Osaka, Japan.
SOURCE: Protein engineering, (1996 Oct) 9 (10) 857-67.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970602
Last Updated on STN: 19970602
Entered Medline: 19970522

AB Three mutants of Escherichia coli ribonuclease HI, in which an invariant acidic residue Asp134 was replaced, were crystallized, and their three-dimensional structures were determined by X-ray crystallography. The D134A mutant is completely inactive, whereas the other two mutants, D134H and D134N, retain 59 and 90% activities relative to the wild-type, respectively. The overall structures of these three **mutant**

proteins are identical with that of the wild-type enzyme, except for local conformational changes of the **flexible loops** . The ribonuclease H family has a common active site, which is composed of four invariant acidic residues (Asp10, Glu48, Asp70 and Asp134 in E.coli ribonuclease HI), and their relative positions in the mutants, even including the side-chain atoms, are almost the same as those in the wild-type. The positions of the delta-polar atoms at residue 134 in the wild-type, as well as D134H and D134N, coincide well with each other. They are located near the imidazole side chain of His124, which is assumed to participate in the catalytic reaction, in addition to the four invariant acidic residues. Combined with the pH profiles of the enzymatic activities of the two other mutants, H124A and H124A/D134N, the crystallographic results allow us to propose a new catalytic mechanism of ribonuclease H, which includes the roles for Asp134 and His124.

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L12 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:222588 HCAPLUS
DOCUMENT NUMBER: 135:1854
TITLE: Solution structure of the antiapoptotic protein bcl-2
AUTHOR(S): **Petros, Andrew M.**; Medek, Ales; **Nettesheim, David G.**; Kim, Daniel H.; **Yoon, Ho Sup**; Swift, Kerry; Matayoshi, Edmund D.; Oltersdorf, Tilman; **Fesik, Stephen W.**
CORPORATE SOURCE: Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL, 60064, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(6), 3012-3017
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The structures of two isoforms of Bcl-2 that differ by two amino acids have been determined by NMR spectroscopy. Because wild-type Bcl-2 behaved poorly in solution, the structures were determined by using Bcl-2/Bcl-xL chimeras in which part of the putative unstructured loop of Bcl-2 was replaced with a shortened loop from Bcl-xL. These chimeric proteins have a low pI compared with the wild-type protein and are soluble The structures of the two Bcl-2 isoforms consist of 6 α-helixes with a hydrophobic groove on the surface similar to that observed for the homologous protein, Bcl-xL. Comparison of the Bcl-2 structures to that of Bcl-xL shows that although the overall fold is the same, there are differences in the structural topol. and electrostatic potential of the binding groove. Although the structures of the two isoforms of Bcl-2 are virtually identical, differences were observed in the ability of the proteins to bind to a 25-residue peptide from the proapoptotic Bad protein and a 16-residue peptide from the proapoptotic Bak protein. These results suggest that there are subtle differences in the hydrophobic binding groove in Bcl-2 that may translate into differences in antiapoptotic activity for the two isoforms.

CC 6-3 (General Biochemistry)
ST bcl2 alpha helix conformation peptide hydrophobicity binding
IT Hydrophobicity
(bcl-2 hydrophobic binding to peptides)
IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(bcl-2; solution structure of the antiapoptotic protein bcl-2)
IT α-Helix
(solution structure of the antiapoptotic protein bcl-2)
IT 300349-67-1 331762-68-6
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(bcl-2 hydrophobic binding to peptides)
REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT